PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/007025	29.06.2004	08.07.2003

International Patent Classification (IPC) or both national classification and IPC
C12P7/66, C12N9/90, C12N9/12, C12N9/04, C12R1/01

Applicant
DSM IP ASSETS B.V.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/007025

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/007025

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/EP2004/007025

The present application describes a method to produce coenzyme Q-10 by transforming microorganisms of the genus Rhodobacter with a set of genes for the mevalonate pathway from a microorganism belonging to the genus Paracoccus. The set of genes for the mevalonate pathway comprises: MvaA (hydroxymethylglutaryl-CoA reductase), Idi (isopentenyl diphosphate isomerase), Hcs (hydroxymethylglutaryl-CoA synthase), Mvk (mevalonate kinase), Pmlc (phosphomevalonate kinase), and Mvd (diphosphomevalonate decarboxylase).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 02/099095 A (LOPEZ-ULIBARRI RUAL ; ROCHE VITAMINS AG (CH); BERRY ALAN (CH); HUEMBEL) 12 December 2002

D2: WO 02/10398 A (HAHN FREDERICK M ; KUEHNLE ADELHEID R (US)) 7 February 2002

D3: YOSHIDA H ET AL: "PRODUCTION OF UBIQUINONE-10 USING BACTERIA" JOURNAL OF GENERAL AND APPLIED MICROBIOLOGY, vol. 44, no. 1, 1998, pages 19-26

1 Claims 1 - 9 formally meet the requirements of Article 33(2) PCT because their subject-matter was not disclosed in the available prior art.

2 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 - 9 does not involve an inventive step in the sense of Article 33(3) PCT.

The document D1 is regarded as being the closest prior art to the subject-matter of claims 1 - 9, and discloses (the references in parentheses applying to this document): a method that uses the genes of the mevalonate pathway derived from Paracoccus sp. R114 (in the present application also named P. zeaxanthinifaciens) for improving isoprenoid production; the set of genes for the mevalonate pathway comprises: MvaA (hydroxymethylglutaryl-CoA reductase), Idi (isopentenyl diphosphate isomerase), Hcs (hydroxymethylglutaryl-CoA synthase), Mvk (mevalonate kinase), Pmlc

(phosphomevalonate kinase), and Mvd (diphosphomevalonate decarboxylase) (the whole document).

The subject-matter of the present application differs from the subject-matter of document D1 by the use of microorganisms of the genus Rhodobacter.

The problem to be solved by the present invention may therefore be regarded as providing an alternative host for the production of isoprenoids using genes of the mevalonate pathway.

The solution proposed by the present application is the provision of a microorganism of the genus Rhodobacter transformed with MvaA (hydroxymethylglutaryl-CoA reductase), Idi (isopentenyl diphosphate isomerase), Hcs (hydroxymethylglutaryl-CoA synthase), Mvk (mevalonate kinase), Pmlc (phosphomevalonate kinase), and Mvd (diphosphomevalonate decarboxylase) from a microorganism belonging to the genus Paracoccus.

D1 discloses further that limited availability of IPP limits the production of isoprenoid compounds (page 5, paragraph 1).
Document D2 discloses the manipulation of genes of the mevalonate pathway (the whole document) and that the presence in cells of an additional biosynthetic pathway for the formation of IPP or IPP and DMAPP - by providing a heterologous host with the entire mevalonate pathway or the entire mevalonate pathway plus an additional orf for IPP isomerase - enhances the production of isoprenoid compounds (example 14).

The teaching of D1 or D2 would have been an incentive for the person skilled in the art to use genes of the mevalonate pathway also in other (heterologous) hosts in order to improve isoprenoid production.

In doing so the person skilled in the art would have found Rhodobacter to be a host of choice, in particular if the isoprenoid to be produced is coenzyme Q-10, for the following reasons:
Microorganisms of the genus Rhodobacter are able to produce IPP and are known to be excellent producers of coenzyme Q-10 (e.g. D3).
At the same time the gene cluster for the mevalonate pathway is lacking in Rhodobacter (D1: page 4, paragraph 1). This means that addition of the mevalonate

pathway to Rhodobacter by transformation of the respective genes would add an additional IPP-producing pathway to it that -based on the teaching of D2- increases isoprenoid production.

Therefore, the subject-matter of claims 1 - 9 does not involve an inventive step in the sense of Article 33(3) PCT.

3 The subject-matter of claims 1 - 9 is susceptible of industrial application (Article 33(4) PCT).

Aside from the above-mentioned objections, the following objections/remarks are made:

4 The application does not meet the requirements of Article 6 PCT, because claims 1,3-5,7-9 are not clear. It is not clear which genes are comprised in the expression "mevalonate operon". This objection may be overcome by listing the genes as described on page 5, paragraph 4, of the present application.

5 It is not at present apparent which part of the application could serve as a basis for a new, allowable claim. Should the applicant nevertheless regard some particular matter as patentable, an independent claim should be filed taking account of Rule 6.3(b) (I), (ii) PCT. The applicant should also indicate in the letter of reply the difference of the subject-matter of the new claim vis-à-vis the state of the art and the significance thereof.

6 When filing amended claims the applicant should at the same time bring the description into conformity with the amended claims. Care should be taken during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Articles 19(2) and 34(2) (b) PCT).

7 In order to facilitate the examination of the conformity of the amended application with the requirements of Articles 19(2) and 34(2) (b) PCT, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.